ALLIA.143CP2

OIPE JC37 JUL 05 2001 PATENT & TRADEMARK OFFICE

PATENT

#18 7/14/01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	H. Zaghouani
Appl. No.	:	09/111,123
Filed	:	July 6, 1998
For	:	COMPOUNDS, COMPOSITIONS AND METHODS FOR THE ENDOCYTIC PRESENTATION OF INNUMOSUPPRESSIVE FACTOR
Examiner	:	P. Nolan

Group Art Unit 1644

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on

July 2, 2001
(Date)

Daniel Hart
Daniel Hart, Reg. No. 40,637

RECEIVED
JUL 11 2001
TECH CENTER 1600/2900

DECLARATION UNDER 37 C.F.R §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

1. This Declaration is being submitted to demonstrate that the compositions claimed in the above-identified application permanently eliminate the symptoms of autoimmune disease in all subjects treated by preventing T cell activation. Furthermore, this declaration demonstrates that the elimination of the symptoms of autoimmune disease upon treatment with the compositions claimed in the above-identified application is not a consequence of increased half-life of the claimed compositions, that neither the compositions disclosed in Bona nor the compositions disclosed in Kuchroo or Karin are capable of preventing T cell activation as do the compositions claimed in the above-identified patent application, and that the immunoglobulins comprising a T cell receptor antagonist are generally effective in treating autoimmune diseases.

2. I am an inventor on the above-identified application and am familiar with the specification and prosecution history.

3. I am skilled in the fields of immunology and molecular biology as evidenced by the accompanying curriculum vitae (Exhibit A).

4. The autoimmune disease experimental allergic encephalomyelitis (EAE), an animal model for multiple sclerosis, was induced in 6-8 week old mice by injecting them subcutaneously in the footpads with 100 μg of PLP1, a fragment of myelin proteolipid protein. The PLP1 peptide was administered in 200 μl incomplete Freund adjuvant IFA/PBS solution containing 200 μg *Mycobacterium tuberculosis* H37Ra and 100 μg of free PLP1 peptide. Six hours later, the mice were given $5X10^9$ inactivated *Bordetella pertussis*. A second injection of *Bordetella pertussis* was given after 48 hours. Subsequently, the mice were scored daily for clinical signs of EAE as follows: 0 no clinical score, 1 loss of tail tone, 2 hindlimb weakness, 3 hindlimb paralysis, 4 forelimb paralysis, and 5 moribund or death.

When signs of paralysis became apparent, the mice were treated with 500 μg of Ig-PLP-LR, an immunoglobulin containing the T cell receptor antagonist PLP-LR therein, on days 9, 13, and 17 post disease induction. Control mice received 500 μg of Ig-W, the parental immunoglobulin of Ig-PLP-LR which does not have a peptide inserted therein, on days 9, 13 and 17 post disease induction. The mice were then scored daily for clinical signs of EAE.

5. Exhibit B illustrates the results of the above experiment. Each point represents the mean clinical score of 8 mice, with closed circles indicating the results obtained in mice receiving Ig-PLP-LR and open circles indicating the results obtained in the control mice. As depicted in Exhibit B, the mice treated with the control immunoglobulin developed EAE and did not recover for the 120 day period of observation. However, all of the mice treated with Ig-PLP-LR developed milder symptoms during the initial stages of treatment, fully recovered by 50 days post disease induction, and never relapsed.

The results of this experiment indicate that treatment with immunoglobulins having a T cell receptor antagonist therein permanently eliminated disease symptoms in all of the animals tested.

6. The elimination of the symptoms of autoimmune disease upon treatment with the compositions claimed in the above-identified application is not a consequence of increased half-life of the claimed compositions. As indicated in Exhibit B, mice suffering from EAE fully recovered from this autoimmune disease within 50 days of treatment with the claimed compositions and did not relapse for the duration of the experiment (120 days from the

administration of the claimed compositions). The experiment of Exhibit B demonstrates that although a period of 15 weeks elapsed between the last administration of the compositions claimed in the above-identified application and the termination of the experiment, the mice did not develop disease symptoms.

7. The effectiveness of treatment with the compositions claimed in the above-identified application is unlikely to be a result of increased half life resulting from insertion of the T-cell antagonist into an immunoglobulin backbone. The half life of immunoglobulins in 6-8 week old mice such as those used in the experiment of Exhibit B is on the order of 4.5 days. (See Takemori et al. Immunological Review 79: 103-117 (1984), provided herewith as Exhibit C). Thus, since the amount of immunoglobulin present in the subjects after 15 weeks is negligible (approximately 2^{25} times lower than the originally administered amount), it is unlikely that a sufficient amount of the composition remains to provide direct protection. Rather, as discussed in more detail below, the effectiveness of the claimed compositions is most likely due to a permanent inactivation of the T cells directed against the antigen responsible for the autoimmune disease.

8. The compositions claimed in the above-identified patent application permanently eliminated the symptoms of autoimmune disease in subjects which were suffering from autoimmune disease prior to the administration of the claimed compositions.

The mice treated with the claimed composition in Exhibit B were approximately 2 months old at the time the claimed compositions were administered. Thus, at the conclusion of the experiment (120 days from the administration of the claimed compositions) the mice were approximately six months old. As indicated in Endoh, M. et al., Journal of Neuroimmunology 29: 21-27, which is provided herewith as Exhibit D, mice have poor susceptibility to EAE once they reach the age of 24-27 weeks (6 months). Accordingly, the fact that the mice treated with the claimed compositions did not exhibit disease symptoms at 6 months of age indicates that they were permanently cured of the disease, since at that age they are no longer susceptible to the disease.

9. Neither the compositions disclosed in Bona nor the compositions disclosed in Kuchroo or Karin are capable of inactivating T cells as do the compositions claimed in the above-identified patent application. The compositions of the present invention prevent activation of T cells directed against proteolipid protein as follows. Immunoglobulins containing a T cell

receptor antagonist derived from the proteolipid protein are internalized into antigen presenting cells via their interaction with the Fc receptor. Inside the cell, the immunoglobulins move to the endosomes where antagonist peptides are cleaved from the immunoglobulin and bind to newly synthesized MHC molecules. The complexes between the antagonist peptides and the MHC molecules move to the cell surface where they engage autoreactive T cells. The interaction between the antagonist/MHC complexes and the autoreactive T cells reduces cytokine production, thereby inactivating autoreactive T cells.

This mechanism of action is documented in the accompanying article by Legge et al., J. Exp. Med. 185: 1043-1053 (1997) provided herewith as Exhibit E. Experiments using immunoglobulins containing the T cell receptor antagonist PLP-LR demonstrated that these compositions reduced proliferation of T cells *in vitro* (See pages 1047-1048 and Figure 5 of Exhibit E) and *in vivo* (See pages 1048 and Figure 8 of Exhibit E).

Furthermore, the inactivation of T cells does not occur by a competitive mechanism in which the T-cell antagonist occupies MHC Class II molecules and prevents the antigenic peptide from binding thereto. This is evidenced by the fact that immunoglobulins containing the PLP-2 peptide, which is not a T cell receptor antagonist, inserted therein did not prevent T cell activation, while immunoglobulins containing the T cell receptor antagonist PLP-LR did prevent T cell activation. (See page 1048 and Figure 6 of Exhibit E).

The compositions which had been actually prepared and which were discussed in Bona were compositions in which an immunogenic peptide was inserted into an immunoglobulin backbone. Thus, the goal of these compositions was to stimulate an immune response (i.e. activate T cells) rather than to inhibit an immune response (i.e. prevent T cell activation) as do the compositions of the present invention.

While Bona speculates that self antigens could be introduced into immunoglobulin backbones, there is no mention of inserting T cell antagonists into the immunoglobulin backbone. Furthermore, in Bona's discussion of self antigens he states "In the later case cases, it is possible that the Ig bearing epitopes of self antigen will be more efficient for peptide competition therapy envisioned as a novel therapeutic approach of autoimmune disease." This statement indicates that Bona hypothesized that the speculated immunoglobulins containing self antigens would be internalized into the cells, bind MHC proteins inside the cells, and be transported to the surface of the cells such that all MHC proteins on the surface of the cells

would be occupied by the self antigen and would be unavailable for binding the pathogenic peptide. This mechanism is unlikely to work because MHC molecules and pathogenic peptides are synthesized continuously in unlimited amounts. Accordingly, in contrast to Bona's hypothesis, complexes between the MHC molecules and the pathogenic peptides would in fact be formed and translocate to the surfaces of the antigen presenting cells. Bona's hypothesis was ruled out in Figure 6 of Exhibit E. Accordingly, Bona did not conceive of the above mechanism whereby immunoglobulins containing T cell antagonists (note that Bona mentions immunoglobulins containing antigens or self antigens but makes no mention of immunoglobulins containing T cell antagonists) prevent T cell activation.

The compositions disclosed in Kuchroo or Karin are incapable of preventing T cell activation. Kuchroo and Karin disclose peptides which function as T cell receptor antagonists. Since the peptides of Kuchroo and Karin are not embedded in an immunoglobulin backbone, they are not internalized via the mechanism described above with respect to the compositions of the present invention. Accordingly, the peptides of Kuchroo and Karin cannot bind to newly synthesized MHC molecules to prevent T cell activation via the mechanism utilized by the compositions of the present invention.

10. Immunoglobulins containing T cell receptor antagonists derived from proteins other than proteolipid protein were also effective in treating autoimmune disease. In particular, the following experiment demonstrates that immunoglobulins containing a T cell receptor antagonist derived from myelin basic protein (MBP) were effective in suppressing experimental allergic encephalomyelitis (EAE) induced by MBP87-99 peptide.

The amino acid sequence 87-99 (VHFFKNIVTPRTP) of myelin basic protein (MBP87-99) is encephalitogenic and induces experimental allergic encephalomyelitis when injected into SJL/J mice emulsified in complete Freund's adjuvant (CFA). The EAE induced by MBP87-99 is less severe than that induced by proteolipid protein. EAE induced by MBP87-99 is characterized by a single episode of disease followed by complete recovery as opposed to the relapse and remitting EAE induced by proteolipid protein.

For simplicity purposes MBP87-99 peptide is referred to herein as MBP3 peptide. An altered peptide was generated from MBP3 by substituting the proline in position 96 with alanine. This altered peptide, designated MBP3A (VHFFKNIVTARTP), functions as a T cell antagonist

and suppresses passive disease transferred into mice by a pathogenic T cell clone specific for MBP3 (Brock et al., Nature 379: 343-346 (1996), provided herewith as Exhibit F).

A nucleotide sequence encoding MBP3A was inserted into the variable region of the 91A3 immunoglobulin heavy chain and this chimeric heavy chain was transfected into SP2/0 B cell line along with a nucleotide sequence encoding the parental 91A3 immunoglobulin light chain. The resulting immunoglobulin containing the MBP3A peptide therein, designated Ig-MBP3A, was then purified and tested for suppression of EAE induced by MBP3 peptide as follows.

Seven week old SJL/J mice were induced for experimental allergic encephalomyelitis (EAE) with MBP3. Induction of EAE was carried out by subcutaneous injection in the footpads and at the base of the limbs and tail with a 200 μl incomplete Freund adjuvant (IFA)/PBS (v/v) solution containing 200 μg *Mycobacterium tuberculosis* H37Ra and 100 μg of free MBP3 peptide. Six hours later the mice were given intravenously 5 x 10^9 inactivated *Bordetella pertussis* (Bioport, Lansing, MI). A second injection of *B. pertussis* was given after 48 hours. Subsequently, the mice were scored daily for clinical signs of EAE as follows: 0, no clinical score; 1, loss of tail tone; 2, hindlimb weakness; 3, hindlimb paralysis; 4, forelimb paralysis; and 5, moribund or death.

When sign of paralysis became apparent one group was treated with Ig-MBP3A (indicated by circles in Exhibit G provided herewith), another group with the control Ig-W not including any MBP peptide (indicated by squares in Exhibit G), and a third group was left untreated (Nil, indicated by triangles in Exhibit G). The mice were then scored daily for clinical signs of EAE. Each point represents the mean clinical score of 7 mice. Ig-MBP3A is the Ig chimera carrying the partial antagonist peptide, MBP3A, and Ig-W is the parental Ig not carrying any PLP or other peptide which was used as a control. The treatment consists of three intraperitoneal injections of 200 μg of Ig-MBP3A or Ig-W on days 11, 16, and 21 post disease induction totaling 600 μg for all injections.

As indicated in Exhibit G, the untreated mice developed a mild monophasic EAE characteristic of disease induced with MBP3 peptide. The mice treated with the control Ig-W developed clinical paralysis similar to the untreated mice. However, treatment with Ig-MBP3A prevented the disease from taking a normal course and the mice did not suffer symptoms more severe than exceed a mild loss of tail tone for the entire 50 day period of clinical assessment.

These results indicate that delivery of immunoglobulins containing T cell receptor antagonists therein down-regulated pathogenic T cells and that such effectiveness is not unique to immunoglobulins containing a T cell receptor antagonist derived from proteolipid protein but is a general property of immunoglobulins containing T cell receptor antagonists.

11. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: July 2, 2001

By: [signature]
Habib Zaghouani

S:\DOCS\SGJ\SGJ-1182.DOC
062001

CURRICULUM VITAE

HABIB ZAGHOUANI, PH.D.

The University of Tennessee	Office	(865) 974-4025
Department of Microbiology	Lab	(865) 974-6424
M409 Walters Life Sciences Bldg	Fax	(865) 974-4007
Knoxville, TN 37996-0840	E-mail	hzagh@utk.edu

Education

Ph.D.	1987	Immunology, University of Paris/Cancer Research Institute, Paris, France.
M.S.	1983	Immunology, University of Paris/Pasteur Institute, Paris, France.
B.S.	1981	Biochemistry, University of Paris, Paris, France.

Research experience

2000-present: Associate Professor, Microbiology, The University of Tennessee, Knoxville (recently promoted to Associate Professor, Official title pending approval by the President of the University)

1994-2000: Assistant Professor, Microbiology, The University of Tennessee, Knoxville.

1990-1994: Research Assistant Professor, Microbiology, Mount Sinai School of Medicine, New York.

1987-1989: Postdoctoral Fellow, Microbiology, Mount Sinai School of Medicine, New York. Advisor: Dr. Constantin A. Bona.

1983-1987: Graduate Research Assistant, Immunology, Cancer Research Institute, Paris, France. Mentor: Dr. Marc Stanislawski.

1981-1983: Graduate Research Assistant, Immunology, Pasteur Institute, Paris, France. Director: Dr. Arthur Dony Strosberg.

Teaching Experience

1992-1994: 600-level Immunology course, 3 credit hours, 6 lecture contact hours, 10 students, Spring Semester, Microbiology, Mount Sinai School of Medicine, New York.

1995-present: Microbiology 430 (Immunology), 3 credit hours, 45 lecture contact hours, 100-120 students, Fall Semester, Microbiology, The University of Tennessee, Knoxville.

1995-present: Co-direct Microbiology 602 (Microbial Pathogenesis Journal Club), 1 credit hour, 15 lecture contact hours, 10-15 students, Fall Semester, Microbiology, The University of Tennessee, Knoxville.

1995-present: Co-direct Microbiology 603 (Immunology Journal Club), 1 credit hours, 15 lecture contact hours, 10-15 students, Spring Semester, Microbiology, The University of Tennessee, Knoxville.

1995-Present: Microbiology 401 (Undergraduate Research), 3 credit hours, 1-2 students per semester, Microbiology, The University of Tennessee, Knoxville.

1998: Microbiology 630 (Topics in Immunology), 3 credit hours,10 lecture contact hours, 20 students, Spring Semester, (Seminar Series) Microbiology, The University of Tennessee, Knoxville.

1998: Microbiology 493 (Independent Study in Immunology), 6 students, 10 lecture contact hours, Spring Semester, Microbiology, The University of Tennessee, Knoxville.

Honors and Awards

1999: Chancellor's nomination for Howard Hughes Medical Institute Assistant Investigator Appointment, The University of Tennessee, Knoxville (application pending).

1999: Biological Equipment Award,Office of Research Adminstration/Science Alliance/Genome Science and Technology/Division of Biology, The University of Tennessee, Knoxville.

1999: Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.

1999: Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of research Administration, The University of Tennessee, Knoxville.

EXHIBIT A

1998:	Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee., Knoxville.
1998:	Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of Research Administration, The University of Tennessee, Knoxville.
1997:	Biological Equipment Award, Office of Research Adminstaration/Science Alliance/ Division of Biology/ Department of Microbiology, The University of Tennessee, Knoxville.
1997:	Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of Research Administration, The University of Tennessee, Knoxville.
1990:	Research Excellence Award, Alliance Pharmaceutical Corporation. San Diego, CA.
1987-1988:	Scientist Exchange Award (Postdoctoral Fellowship), French Cancer Society , Paris, France.
1984-1987:	Graduate Student Scholarship, French Cancer Society, Paris, France.

Graduate Students and Postdoctoral Research Associates

1990-1992:	Honor Research Thesis	Daniel Goldstein, Mount Sinai School of Medicine, NY.
1995-present:	Ph.D. (expected 02/00)	Booki Min, The University of Tennessee, Knoxville.
1996-1998:	M.S.	Aimee Cestra, The University of Tennessee, Knoxville.
1996-present:	Ph.D (expected 09/00)	Kevin L. Legge, The University of Tennessee, Knoxville.
1997-present:	M.S. (expected 03/00)	Christopher Pack, The University of Tennessee, Knoxville.
1998-present:	Ph.D. (in progress)	Randal Gregg, The University of Tennessee, Knoxville.
1998-present:	M.D., Ph.D. (Postdoc)	Lequn Li, The University of Tennessee, Knoxville.
1999-present:	M.S. (in progress)	Jacque Caprio, The University of Tennessee., Knoxville.
2000-present:	Ph.D., (in progress)	Jeremiah Bell, The University of Tennessee, Knoxville.

Graduate Degree Committees

1995-1998:	M.S. Microbiology	Jack McPherson, The University of Tennessee, Knoxville.
1996-1998:	M.S. Microbiology	Aimee Cestra, The University of Tennessee, Knoxville.
1996-1999:	Ph.D. Microbiology	Sangjun Chun, The University of tennessee, Knoxville.
1997-1999:	M.S. Microbiology	Kristin Lavander, The University of Tennessee, Knoxville.
1997-1999:	M.S. Microbiology	Amanda Royer, The University of Tennessee, Knoxville.
1996-present:	Ph.D. Microbiology	Booki Min, The University of Tennessee, Knoxville.
1997-present:	Ph.D. Microbiology	Kevin Legge, The University of Tennessee, Knoxville.
1998-present:	Ph.D. Microbiology	Shilpa Desphande, The University of Tennesee, Knoville.
1999-present:	M.S. Microbiology	Christopher Pack, The University of Tennessee, Knoxville.

Professional Service

1992-present:	Editorial board member: *Viral Immunology*
1992-present:	Reviewer: *The Journal of Immunology*
1993-present:	Reviewer: *Molecular Immunology*
1993-present:	Reviewer: *Autoimmunity*
1995-present:	Member of The Graduate Student Recruitment Committee, Department of Microbiology, The University of Tennessee, Knoxville.
1996-present:	Reviewer: *Infection and Immunity*
1996-present:	Reviewer: *Cellular Immunology*
1998:	Member of Faculty Search Committee, Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennesee, Knoxville.
1999:	Panel Member: NIH/NCI, Small Business Innovation Research (SBIR)/Small Business Technology Transfer (STTR) Grant program. Flexible system to advance innovative research for cancer drug discovery by small business panel.

Professional Membership

1992-present:	Member of the American Association for the Advancement of Science.
1992-present:	Member of the American Association of Immunologists.
1998-pr sent:	Member of the Society for Neuroscience

Invited Speaker

2000	Division of Research, Alliance Pharmaceutical Corporation, San Diego, CA
1999:	Division of Research, Alliance Pharmaceutical Corporation, San Diego, CA.
1999:	Keystone Symposia, Immunogenetics of Human Disease, MHC/TCR & Peptide, Taos, NM.
1999:	Immunobiology Center, Mount Sinai School of Medicine, New York, NY.
1999:	Department of Microbiology, The University of Tennessee, Knoxville, TN.
1998:	Division of Research, Alliance Pharmaceutical Corporation, San Diego, CA.
1997:	Division of Research, Alliance Pharmaceutical Corporation, San Diego, CA.
1997:	Center for Neurologic Diseases, Harvard Medical School, Boston, MA.
1997:	Department of Microbiology, Vanderbilt School of Medicine, Nashville, TN.
1996:	Department of Microbiology, University of Tennessee, Knoxville, TN.
1996:	Division of Research, Alliance Pharmaceutical Corporation, San Diego, CA.
1995:	Department of Neurology, University of Alabama, Birmingham, AL.
1995:	Department of Biochemistry, Molecular & Cell Biology, The University of Tennessee, Knoxville, TN.
1994:	The Molecular and Immunological basis of Development of Vaccines, Fondation Merieux, Annecy, France.
1993:	Department of Microbiology, College of Biological Sciences, Columbus, OH.
1993:	Department of Microbiology, Dartmouth School of Medicine, Hannover, NH.
1993:	Unite d'Imunologie Cellulaire et Cinique, Institut Curie, Paris, France.
1993:	Research Division, Southwest Foundation for Biomedical Research, San Antonio, TX.
1993:	Department of Microbiology, Evansville Center for Medical Education, Evansville, IN.
1990:	International Conference on Cellular and Molecular Aspect of Self Reactivity and Autoimmune Diseases,Taormina, Italy.
1992:	Department of Microbiology, Medical College of Pennsylvania, Philadelphia, PA.
1992:	Federation of American Societies for Experimental Biology, Anaheim, CA.
1991:	Federation of American Societies for Experimental Biology, Atlanta, GA.
1990:	Centre de Recherche en Virologie, Institut Armand-Frappier, Laval, Quebec, Canada.

Research Grant Support

Active

1). RG2967A2/1, National Multiple Sclerosis Society, April 99 - March 2002. Down-regulation of encephalitogenic T cells. Direct cost: $290,934/3years.

2). RO1101564, Astral Inc:., March 95 - February 2001. A novel approach to delete encephalitogenic T cells. Direct cost: $365,000/6 years.

3). 1RO1NS/AI37406, National Institutes of Health, January 2000- December 2002. Modulation of autoreactive T cells. Direct cost: $491,415/3years.

Pending

1). National Institutes of Health, Regulation of Neonatal Immunity . Direct Cost: $908,265/5 years.

Previous Support

1). RG2778A1/1, National Multiple Sclerosis, April 96 - March 1999. A deletional strategy for encephalitogenic T cells. Direct cost: $ 252,573/3 years.

2). RO1101572, Astral Inc:., September 97- August 99. Generation of human Ig chimeras carrying wild type or antagonist forms of myelin peptides. Direct cost:$ 248,500/2 years

PUBLICATIONS

Manuscripts submitted for publication in peer-review Journals

40. Min, B., Legge, K. L., Li, L., Caprio, J. C., Gregg, R. K., Bell, J. J., and Zaghouani, H. (2000). Defective up-regulation of IL-2 receptor alpha chain underlies interferon-gamma mediated T cell anergy. Submitted for publication.

39. Legge, K. L., Min, B., Caprio, J. C., Li, L., Gregg, R. K., Bell, J. J., and Zaghouani, H. (2000). Coupling of peripheral tolerance to endogenous IL-10 promotes effective modulation of myelin-activated T cells and ameliorates experimental allergic encephalomyelitis. Being revised for J. Exp. Med.

38. Day, R. B., Okada, M., Ito, Y., Tsukada, K., Zaghouani, H., Shibuya, N., and Stacey, G. (2000). Identification of a high affinity binding site of N-acetylchitooligosaccharides localized in the plasma membrane of soybean. Submitted for publication_.

Manuscripts published in peer-review journals

37. Anderson, A. C., Nicholson, L. B., Legge, K. L., Turchin, V., Zaghouani, H., and Kuchroo, V. K. (2000). High frequency of auto-reactive myelin proteolipid protein (PLP)-specific T cells in the periphery of naïve mice: mechanisms of selection of the self-reactive repertiore. J. Exp. Med. In press.

36. Min, B., Legge, K. L., Caprio, J. C., Li, L., Gregg, R., and Zaghouani, H. (2000). Differential control of neonatal tolerance by antigen dose versus extended exposure and adjuvant. Cell. Immunol. In press.

35. Min, B., Legge, K. L., Li, L., Caprio, J. C., Pack, C. D., Gregg, R., McGavin, D., Slauson, D., and Zaghouani, H. (1999). Neonatal tolerant immunity for vaccination against autoimmunity. Intl. Rev. Immunol. In press .

34. Legge, K. L., Min, B., Pack, C. D., Caprio, J. C., and Zaghouani, H. (1999). Differential presentation of an altered peptide within fetal central and peripheral organs supports an avidity model for thymic T cell development and implies a peripheral re-adjustment for activation. J. Immunol._162:5738-46.

33. Min, B., Legge, K. L., Pack, C. D. and Zaghouani, H. (1998). Neonatal exposure to a self peptide-Ig chimera circumvents the use of adjuvant and confers resistance to autoimmune disease by a novel mechanism involving IL-4 lymph node deviation and INFγ-mediated splenic anergy. J. Exp. Med. 188:2007-17.

32. Legge, K. L., Min, B., Cestra, A.E., Pack, C. D., and Zaghouani, H. (1998). T cell receptor agonist and antagonist exert in vivo cross-regulation when presented on immunoglobulins. J. Immunol. 161:106-11.

31. Legge, K. L., Min, B., Potter, N.T., and Zaghouani, H. (1997). Presentation of a T cell receptor antagonist peptide by immunoglobulins ablates activation of T cells by a synthetic peptide or protein requiring endocytic processing. J. Exp. Med. 185:1043-53.

30. Brumeanu, T-D, Dehazya, P., Wolf, I., Bot, A., Bona, C., and Zaghouani, H. (1996). Engineering of double antigenized Igs carrying B and T cell epitopes. Immunotechnology 2:85-95.

29. Brumeanu, T-D., Zaghouani, H., and Bona, C. (1995). Purification of antigenized immunoglobulins derivatized with monomethoxypolyethylene glycol. J. Chromatogr. 696:219-25.

28. Brumeanu, T-D., Zaghouani, H., Elahi, I., Daian, C. and Bona, C. (1995). Derivatization with monomethoxypolyethylene glycol of Igs expressing viral epitopes obviates adjuvant requirement. J. Immunol. 154:3088-95.

27. Zaghouani, H., Anderson, S., Sperber, K. E., Daian, C., Kennedy, R. C., Mayer, L. and Bona, C. (1995). Induction of antibodies to the human immunodeficiency virus type 1 by immunization of baboons with immunoglobulin molecules carrying the principal neutralizing determinant of the envelope protein. Proc. Natl. Acad. Sci.USA. 92:631-35.

26. Bona, C., Brumeanu, T-D and Zaghouani, H. (1994). Immunogenicity of microbial peptides grafted in self immunoglobulin molecules. Cell. Mol. Biol. 40 (suppl):21-30.

25. Brumeanu, T-D., Swiggard, W. J., Steinman, R. M., Bona, C., and Zaghouani, H. (1993). Efficient loading of identical peptide onto class II molecules by antigenized immunoglobulin and PR8 virus. J. Exp. Med. 178:1795-99.

24. Brumeanu, T-D., Kohanski, R., Bona, C., and Zaghouani, H. (1993). A sensitive method to detect defined peptide among those eluted from murine MHC class II molecules. J. Immunol. Meth. 160:65-71.

23. Kuzu, Y., Kuzu, H., Zaghouani. H., and Bona, C. (1993). Priming of CTLs at various stages of ontogeny with transfectoma cells expressing a chimeric Ig heavy chain gene bearing an influenza virus nucleoprotein. International. Immunol. 5:1301-07.

22. Zaghouani, H., Kuzu, Y., Kuzu, H., Swigard, W., Steinman, R., and Bona, C. (1993). Contrasting efficacy of presentation by major histocompatibility complex class I and class II products when peptides are administered within a common protein carrier, self immunoglobulin. Eur. J. Immunol. 23:2746-50.

21. Penney, C. L., Ethier, D., Dionne, G., Nixon-George, A., Zaghouani, H., Michon, F., Jennings, H., and Bona, C. (1993). Further studies on the adjuvanticity of stearyl Tyrosine and ester analogues. Vaccine. 11:1129-1134.

20. Kuzu, H., Kuzu, Y., Zaghouani, H., and Bona, C. (1993). In-vivo priming effect during various stages of ontogeny of an influenza virus nucleoprotein derived peptide. Eur. J. Immunol. 23:1397-1400.

19. Zaghouani, H., Steinman, R., Nonacs, R.,. Shah, H., Gerhard, W. and Bona, C. (1993). Efficient presentation of a viral T helper epitope expressed in the CDR3 region of a self immunoglobulin molecule. *Science*. 259:224-27.

18. Shengqiang, L., Polonis, V., Isobe, H., Zaghouani, H., Guinea, R., Moran, T., Bona, C., and Palese, P. (1993). Chimeric influenza virus induces neutralizing antibodies and cytotoxic T cells against human immunodeficiency virus type 1. *J. Virol.* 67:6659-66.

17. Zaghouani, H., Kuzu, Y., Kuzu, H., Mann, N., Daian, C., and Bona, C. (1993). Engineered immunoglobulin molecules as vehicles for T cell epitopes. Int. Rev. Immunol. 10:265-77.

16. Hali, B., Zaghouani, H., Daian, C. and Bona, C. (1992). A single amino acid mutation in CDR3 of the 3-14-9 light chain abolished expression of the IDA 10 defined idiotype and antigen binding. J. Immunol. 149:1605-12

15. Nixon, A., Zaghouani, H., Penney, C. L., Lacroix, M., Dionne, G., Anderson, S., Kennedy, R. C. and Bona, C. A. (1992). Adjuvanticity of stearyl tyrosine on the antibody response to peptide 503-535 from HIV gp160. Viral. Immunol. 5:141-50

14. Zaghouani, H., Krystal, M., Kuzu, H., Moran, T., Shah, H., Kuzu, Y., Schulman, J. and Bona, C. (1992). Cells expressing a heavy chain immunoglobulin gene carrying a viral T cell epitope are lysed by specific cytolytic T cells. *J. Immunol.* 148:3604-09.

13. Zaghouani, H., Goldstein, D., Shah, H., Anderson, S., Lacroix, M., Dionne, G., Kennedy, R. C. and Bona, C. (1991). Induction of antibodies to the envelope protein of the human immunodeficiency virus by immunization with monoclonal anti-idiotypes. Proc. Natl. Acad. Sci. USA. 88:5645-49.

12. Kaushik, A., Mayer, R., Fidanza, V., Zaghouani, H., Lim, A., Bona, C. and Dighiero, G. (1990). LY-1 and V-gene expression among hybridomas secreting natural autoantibody. J. Autoimmunity 3:687-700.

11. Mayer, R., Zaghouani, H., Usuba, O. and Bona, C. (1990). The LY-1 gene expression in murine hybridomas producing autoantibodies. Autoimmunity 6:293-305.

10. Bonilla, F. A., Zaghouani, H., Rubin, M. and Bona, C. (1990). VK gene usage, idiotype expression, and antigen binding among clones expressing the VHX24 gene family derived from naive and anti-id immune Balb/c mice. J. Immunol. 146:616-22.

9. Fidanza, V., Mayer, R., Zaghouani, H., Diliberti, M. A., and Bona, C. (1990). Autoantibodies, LY-1 and immunoglobulin V gene expression in hybridomas obtained from young and old NZB mice. Arthritis & Rheumatism. 33:711-23.

8. Zaghouani, H., Bonilla, F. A., Meek, K. & Bona, C. (1989). Molecular basis for expression of the A48 regulatory idiotype on antibodies encoded by immunoglobulin variable region genes from various families. Proc. Natl. Acad. Sci. USA. 86:2341-45.

7. Zaghouani, H., Fidanza, V. and Bona, C. (1989).The significance of idiotype-anti-idiotype interactions in the activation of self reactive clones. Clin. Exp. Rheumatology. 7/S-3:S19-25.

6. Pinter, A., Honnen, W. J., Tilley, S. A., Bona, C., Zaghouani, H., Zolla-Pazner, S. and Gorny, M. (1989). Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. J. Virol. 63:2674-79.

5. Zaghouani, H., Pene, J., Rousseau, V. and Stanislawski, M. (1988). A new strain specific cross-reactive idiotype with possible regulatory function expressed on Balb/c anti-α(1-3) dextran antibodies. *J. Immunol.* 140:3844-50.

4. Zaghouani, H., and Stanislawski, M. (1987). Regulation of the response to α(1-3) dextran: An anti-dextran associated idiotope of Balb/c mice is also expressed on A/J anti-NIP antibodies. Mol. Immunol. 24:1237-42.

3. Bara, J., Gautier, R., Zaghouani, H. and Decans, C. (1986). Monoclonal antibodies against oncofetal mucin M1 antigens associated with precancerous colonic mucosae. Cancer Res. 46:3983-89.

2. Pene, J., Rousseau, V., Zaghouani, H., Paroutaud, P., Strosberg, D.and Stanislawski, M.(1986). Monoclonal anti-α(1-3) dextran antibodies of Igha Balb/c and Igh^b C.B20 mice display striking similarities. J. Immunol. 137:2319-24.

1. Pene, J., Bekkoucha, F., Desaymard, C., Zaghouani, H., and Stanislawski, M. (1983). Induction of a cross-reactive idiotype dextran-positive antibody response in two $IghC^b$ mouse strains treated with anti-J558 cross-reactive idiotype antibodies. J. Exp. Med. 157:1573-93.

Book Chapters and Reviews

7. Zaghouani, H., and Bona, C. (1992). Stimulation of lymphocytes by anti-idiotypes bearing the internal image of viral antigens. In T Lymphocytes Structure, Function, Choices (eds, Celada, F., and Pernis, B). NATO ASI SERIES, Series A: Life Sciences 233: 121-23.

6. Zaghouani, H., Hall, B., Shah, H. and Bona, C. (1991). Immunogenicity of synthetic peptides corresponding to various epitopes of the human immunodeficiency virus envelope protein. In Adv. Exp. Med. Biol. (ed, Atassi, Z). Plenum Press, New York. 303: 53-62

5. Mayer, R., and Zaghouani, H. (1991). Molecular studies on the contribution of the LY-1 B cell subset to self-reactivity. In Molecular Immunobiology of Self Reactivity. Immunology series. (eds, Bona, C. & Kaushik, A.) Marcel Dekker Publisher, New York. . 55: 61-79

4. Bonilla, F. A., Zaghouani, H. and Bona, C. (1990) Patterns of idiotypic similarity and their structural bases among antibodies specific for foreign and self antigens. In Idiotype in Biology and Medicine. (eds, Carson, D. A., Chen, P. P. and Kipps, T. J.). Prog. Chem. Immunol. Basel, Karger. 48:49-62.

3. Mayer, R., Zaghouani, H., Kaushik, A., Kasturi, K., Fidanza, V. and Bona, C. (1990). The expression of LY-1 and immunoglobulin variable gene families in hybridomas producing autoantibodies of various specificities. In The Molecular Aspects of Autoimmunity. (eds, Farid, N. R. and Bona, C.A.). Academic Press, PP 1-27.

2. Zaghouani, H., Victor-Kobrin, C., Barak, Z., Bonilla, F.A. and Bona, C. (1988). Molecular profile of monoclonal antibodies expressing the A48 regulatory idiotype and having distinct antigenic specificities. Ann. New York. Acad. Sci. 546:248-50.

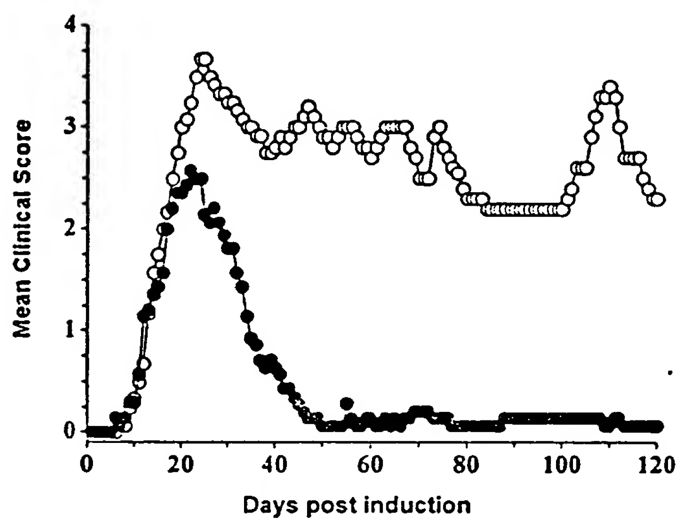
1. Pene, J., Zaghouani, H., and Stanislawski, M. (1984). Regulation of the response to α(1-3) dextran in $IghC^b$ mice. Ann. New York. Acad. Sci. 814:296-304.

Published Abstracts

About 12 abstracts were published in the last 5 years

Patents

1992	Anti-human immunodeficiency virus recombinant antibodies. Constantin Bona and Habib Zaghouani. Ussued in Australia (#18919 672580), Canada (#2107329), and Israel (101602), (April 1992), pending in Europe (#92911196.1) and Japan (#4[1992]510879) .
1994	Patent # 5,969, 109, chimeric antibodies comprising antigen binding sites and B and T cell epitopes, Constantin Bona and Habib Zaghouan. Issued October 19, 1999..
1997	Compound, compositions and methods for the endocytic presentation of immunosuppressive factors. Habib Zagouani. Pending (#08/779,767)



Mean Clinical Score
4
3
2
1
0
0
20
40
60
80
100
120
Days post induction

EXHIBIT B